Haematocrit change in recreational scuba divers following single dive exposure.

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PART ONE : PROTOCOL

Protocol (1) : theoretical background

The study aims to investigate whether haematocrit change occurs in healthy scuba divers in response to a single open water dive at the shallow to medium depths (i.e. less than 30 metres) typically encountered by the recreational scuba diver using air as the breathing gas.

Physiology

For the recreational diver the scuba-diving environment presents numerous reasons for increased fluid loss from the body. Of prime importance is the increased pressure underwater and its even distribution across the body surface; as a result the hydrostatic effect of gravity on the capacitance vessels of the legs is lost, causing an increased venous return from the peripheries to the central circulation. In the face of this relative central fluid overload the release of atrial natriuretic peptide prompts the removal of excess plasma volume by the kidney.[i] Other physiological responses to immersion include peripheral vasoconstriction in response to cold water which further increases central blood pooling and consequent diuresis. In the lungs, dry gas from the scuba tank increases the rate of respiratory water loss.

Packed cell volume or haematocrit can be used as a proxy measure for fluid loss and dehydration[ii]. Based on the above theory, it should be expected that haematocrit would increase upon return to atmospheric pressure since, as the diver decompresses and the circulation to the peripheries is restored, the diuresis which has occurred at depth should now yield a relative hypovolaemia at the surface. Yet although the theory is widely held [iii] there is a lack of evidence from recreational diving studies of the increase in haematocrit which is implied. Nonetheless, rehydration therapy commonly features as a routine intervention in the early treatment of the recreational diving casualty.[iv] [v]

Previous related work

Studies exist showing an increase in haematocrit in response to pressure exposure, both in-water[vi] and in the pressure chamber.[vii] [viii] But neither the prolonged duration of these studies, nor the breathing gases used, nor the depths at which the studies have been conducted (in excess of than 300m for all three examples quoted), can be said to replicate the recreational diving experience.

Studies looking specifically at changes in blood parameters which could be induced by the latter are few. The work that has been done has tended to replicate one isolated aspect of the recreational diving experience, such as pressure, cold shock or water immersion. Positive haematocrit increases have been demonstrated but often the exposure of the study subjects to the variable under test has far exceeded that which would be experienced by the recreational scuba diver.

Concerning pressure the most consistent finding, when pressures typically encountered by the air-breathing scuba diver have been reproduced in the dry environment of a pressure chamber, is that no change in haematocrit occurs, either with repetitive diving[ix] or with single dive exposure.[x] This finding has been reproduced to an equivalent depth of at least 210 feet of sea-water (7.4 ATA).[xi]

Outside the pressure chamber, researchers have examined the physiological response in humans to other features of the diving experience such as breath-holding and the cold shock (at 10 degrees Celsius) of repeated facial immersion[xii xiii]. These studies, performed at atmospheric pressure, have shown that a consistent haematocrit increase of up to 6% occurs in response to these stimuli independently of pressure exposure. This increase is attributed to contraction of the spleen which, as part of a diving reflex in many vertebrates, releases stored erythrocytes into the circulation to maximise oxygen transport during periods of hypoxia. [xiv] Haematocrit increases of 10% have also been observed in professional free divers after a 3 hour period of repetitive breath-hold diving.[xv] But there are no published studies which attempt to replicate these observations in amateur scuba divers with shorter exposure times; indeed, their relevance to the recreational scuba diver is questionable since, equipped with scuba, there should be no requirement to breath hold. In addition the cold shock of immersion may be of smaller significance to a scuba diver in a tropical, warm water environment.

In-water studies, immersing the subject up to the neck in a water bath at thermoneutral temperatures, have also shown increases of haematocrit, especially when the immersion medium is salt rather than fresh water (8.1% and 4.7% respectively[xvi]). Once again, prolonged immersions well in excess of normal scuba-dive exposures have been used to obtain these figures (4 hours in the case of the quoted study). Indeed, the first half-hour or early immersion phase has been associated with a 4% haemodilution of blood volume, with haemoconcentration only commencing after diuresis has begun.[xvii]

Conclusion

In short the evidence surrounding changes in haematocrit in response to recreational air diving is scanty, being mostly derived from findings in other related fields of activity.

Protocol (2) : study design

Aim of study

This study will test for haematocrit change in divers who undergo a typical warm water recreational diving exposure. Volunteer divers will be recruited to provide pre-dive and post-dive samples which will be analysed for haematocrit change. If recruitment patterns allow, the data will also be analysed to see if the overall haematocrit change is duplicated by the sexes individually.

As a secondary aim it is hoped that the collected data can also be analysed for evidence of a difference in the degree of haematocrit change incurred by divers who dive to different depths, or who make dives of differing durations. Without prior knowledge of the survey site operation, however, this section of the study is difficult to plan in advance.

Statistical preparation

Normal values for haematocrit vary depending on the authority. A mean for healthy males of 45% is typical[xviii] and has been used to determine the haematocrit change that may reliably be detected by this study. Given a standard deviation for haematocrit of 2.5 % only 20 subjects will be needed to detect a haematocrit change of 2 points. To detect a 1 point change, however, will require 68 subjects. As males and females have the same variation there will be no need to adjust for sex differences should there be an unequal ratio of male to female volunteers. These volunteer numbers give 90% power to detect these changes using a significance level of 5% and a two-sided paired t-test to analyse the data.

With regard to the subsidiary aim of testing for differing degrees of haematocrit change associated with depth and dive duration it is accepted that this analysis is likely to be under-powered, given that the necessary use of an independent rather than paired t-test will require a sample size of the order of 100 to show a 1% difference in haematocrit at 80% power and 5% significance level. As the number of recruits on the dive site during the 3 months of the project is unlikely to exceed 60 it is probable that no evidence of a significant difference will be found unless the difference in haematocrit change between the groups is large.

Test Subjects

Volunteers will be recruited from divers on the Coral Cay Conservation reef survey project in Fiji. Diving at this site is undertaken typically at depths of up to 30m and is conducted in accordance with the no decompression diving practices of the Professional Association of Dive Instructors (PADI).

A volunteer information sheet will be provided and informed consent obtained – see Appendix 1

Method

Volunteers will provide two blood samples relating to a single dive, the first taken as near as practically possible to descent and the second as soon as possible after surfacing. Blood will be sampled from the ante-cubital fossa in EDTA tubes of the Vacutainer system. The exact timing of sampling will be determined by operational considerations, responsibility for which will rest with the dive site supervisor appointed by Coral Cay Conservation. It is hoped that sampling can occur within 10 minutes of the start and finish of each dive. The depth and duration of each dive will be recorded from the dive computer worn by the diver. These will be provided by the divers themselves or supplied by Coral Cay Conservation. In the interests of standardization all samples will be taken from divers operating on the same dive site.

Following collection, blood samples will be drawn into capillary tubes and centrifuged on the same day in a Hawksley 01410 micro-centrifuge prior to manual reading for haematocrit on a visual sliding plate. Samples will then be placed in a locked sharps bin for later disposal at Nadi mainland hospital.

Processing of data

In order that the analysis should not be influenced by abnormal data, all paired readings will be excluded from analysis where the initial haematocrit exceeds two standard deviations from the same-sex mean in the study population. Normal distribution of sample results will be assessed using a Shapiro-Wilk test. If distributions are normal a paired t-test will be used to compare the pre-dive and post-dive haematocrit values. If distributions are non-normal a non-parametric analysis such as Wilcoxon's test will be used. P-values will be given as a measure of the significance of any changes observed between the paired pre-dive and post-dive haematocrit values. Confidence intervals of 95% will be presented.

Interpretation of results

A significant haematocrit increase over the course of a dive would imply haemo-concentration and so lend weight to the practice of routine rehydration of recreational diving casualties. Likely scenarios to explain haemoconcentration would be splenic contraction resulting in an increased circulating red cell mass and/or a circulating plasma volume reduced by a compression diuresis and increased respiratory losses due to breathing increased volumes of dry air. Proving or refuting the contribution of each of these phenomena would be the subject of further study. A finding of no increase in haematocrit would imply a re-evaluation of the above physiological theory -at least in its application to shallow depth recreational divers. It would call into question the need for routine rehydration in cases of injury to recreational scuba divers.

References

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[xviii] Cardiff and Vale NHS trust *Understanding your blood results* Patient information leaflet 2002

PART TWO : ETHICAL APPROVAL

Approval to pursue this study was granted by the South Devon Regional Ethics committee, following submission of the above protocol, in January 2004. One amendment was made. In order to obtain blood samples as close to the time of descent and surfacing as possible it had originally been envisaged that sampling could be undertaken in a dive boat. It was decided, however, that the presence of sharps could be potentially dangerous in the crowded on-board environment where there would be likely to be much diver movement and activity. In the interests of safety, therefore, it was deemed that only shore-based blood sampling should be practised.

PART THREE : FIELDWORK

This was carried out between the months of February and April 2004 on a dive camp in Fiji operated by Coral Cay Conservation. Divers at this camp undertake reef survey work and perform two dives per day, separated by a minimum lunchtime surface interval of 4 hours, with maximum dive times and depths for each dive being set by the Coral Cay diving staff.

A number of discrete dive sites were being operated from the Fiji camp during the period of this study but in the interests of uniformity a single, shore-dive site was chosen which accorded best with the aim of taking pre-dive and post-dive blood samples as close as possible to the time of dive descent and surfacing. In order that prior knowledge of blood sampling would not influence the divers to change their behaviour volunteers were not generally notified in advance as to the dive on which their blood would be drawn. There was no planned schedule for blood taking; indeed, because the on-site dive plan was drawn up by Coral Cay without input from the researcher, volunteers were simply approached on an ad-hoc basis when they were rostered to dive on the appointed site and when their blood could be conveniently be taken without disruption to the diving activity.

After blood was taken samples were stored in a cool box. Centrifugation was undertaken on the same day in all cases. All sample pairs showing a haematocrit change were re-analysed. In the event of any discrepancy between the first and second tests a third, confirmatory test was undertaken.

The raw data collected appear as a spreadsheet in Appendix 4.

PART FOUR : DATA ANALYSIS

Fieldwork profiles

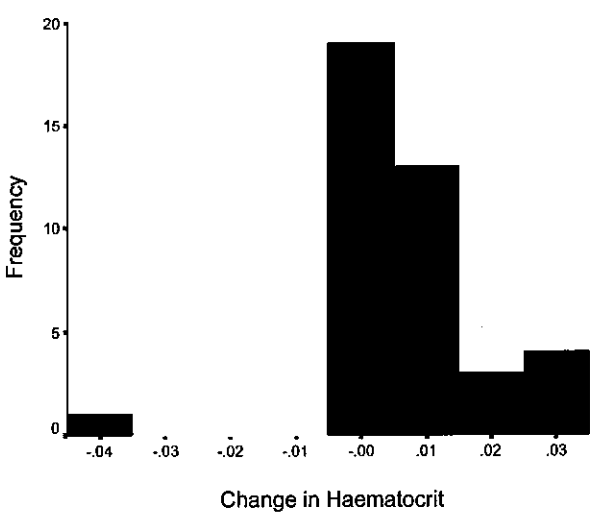
Forty-one participants were recruited, (n = 20 and 21 for males and females respectively) with a mean age of 20.7 (20.8 for females, 20.7 for males).

Pre-dive samples were taken a mean 12.4 minutes before the divers descended (range 8 – 20, standard deviation 3.5 minutes). Post-dive samples were taken a mean 16.2 minutes after surfacing (range 9 – 25, SD 3.7 minutes). The mean dive duration was 39.5 minutes (range 28 – 47, SD 4.5 minutes). The mean maximum depth recorded by divers' computers was 13.6 metres (range 17.4 – 18.1, SD 3.7 metres). The water temperature during the study was of the order of 30° Celsius with a range of approximately 1° over the depths at which the divers were operating.

Data Inclusion

Of the 41 pairs of pre-dive and post-dive samples collected, one post-dive blood sample (from volunteer 23 on the attached spreadsheet) was haemolysed and was thus unsuitable for analysis. Although this pair of samples could not be used for analysis of haematocrit change the pre-dive sample was nonetheless included for calculation of initial pre-dive mean female haematotcrit.

Amongst the 40 remaining sample pairs there was one pair, from volunteer 19, which was thought to be of questionable validity. As shown on the table and histogram below, the sample pairs from 39/40 volunteers either showed no change in haematocrit over the course of the dive, or they showed a change towards haemoconcentration with haematocrit increases between 0.01 and 0.03. This accords with physiological theory. But volunteer 19 showed a haemodilution of 0.04 - the biggest change of the study and in the opposite direction to every other pair. A chart showing the frequency with which varying degrees of haematocrit change was observed has the following appearance :



The same data may be portrayed in tabular form :

Change in Haematocrit		Frequency	
Valid	**-.04**	**1**	
	.00	19	
	.01	13	
	.02	3	
	.03	4	
	Total	40	

On suspicion of a labeling or processing error the data for volunteer 19 were excluded from analysis.

Descriptive statistical analysis was accordingly performed on the 39 remaining sample pairs and one pre-dive sample the matched pair for which was haemolysed. Results were as follows :

Descriptive statistics

	Sex			Statistic	Std. Error
Pre-dive Haematocrit	Female (n=21)	Mean		.3967	.00470
		95% Confidence Interval for Mean	Lower Bound	.3869	
			Upper Bound	.4065	
		Std. Deviation		.02153	
		Minimum		.35	
		Maximum		.43	
		Range		.08	
	Male (n=19)	Mean		.4258	.00574
		95% Confidence Interval for Mean	Lower Bound	.4137	
			Upper Bound	.4378	
		Std. Deviation		.02501	
		Minimum		.37	
		Maximum		.47	
		Range		.10	
Post dive Haematocrit	Female (n=20, haemolysed sample discounted)	Mean		.4020	.00474
		95% Confidence Interval for Mean	Lower Bound	.3921	
			Upper Bound	.4119	
		Std. Deviation		.02118	
		Minimum		.36	
		Maximum		.44	
		Range		.08	

	Male (n=19)	Mean		.4358	.00532
		95% Confidence Interval for Mean	Lower Bound	.4246	
			Upper Bound	.4470	
		Std. Deviation		.02317	
		Minimum		.40	
		Maximum		.48	
		Range		.08	

Calculation of two standard deviations either side of the pre-dive haematocrit means gives a range for males and females of 0.376 – 0.476 and 0.354 – 0.440 respectively. Samples from volunteers 6 and 41 are thus excluded from further analysis since, under the terms of the protocol, the pre-dive haematocrit from these recruits lie outwith two standard deviations of the same-sex mean for the study sample.

The above exclusions having been made, 37 sample pairs (M=18, F=19) were finally included.

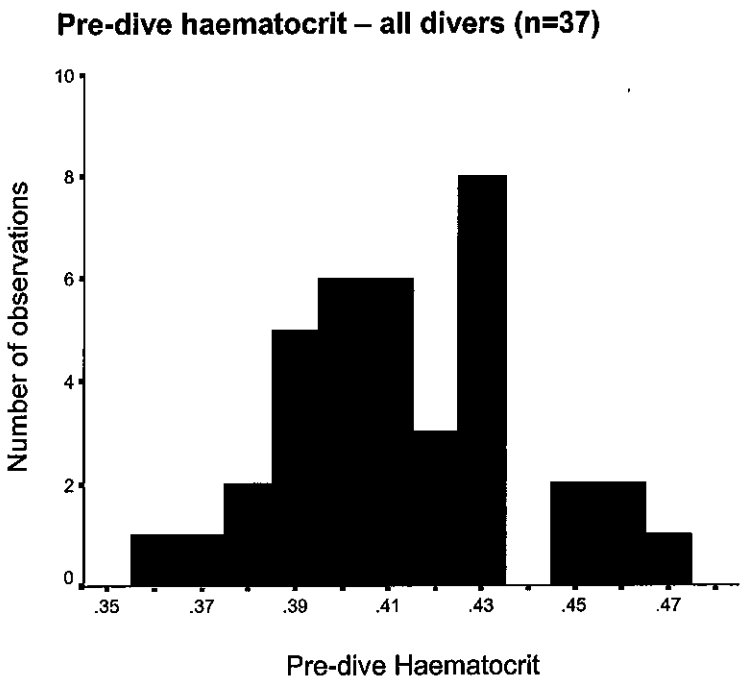
Comparison of pre and post dive haematocrit - ALL DIVERS

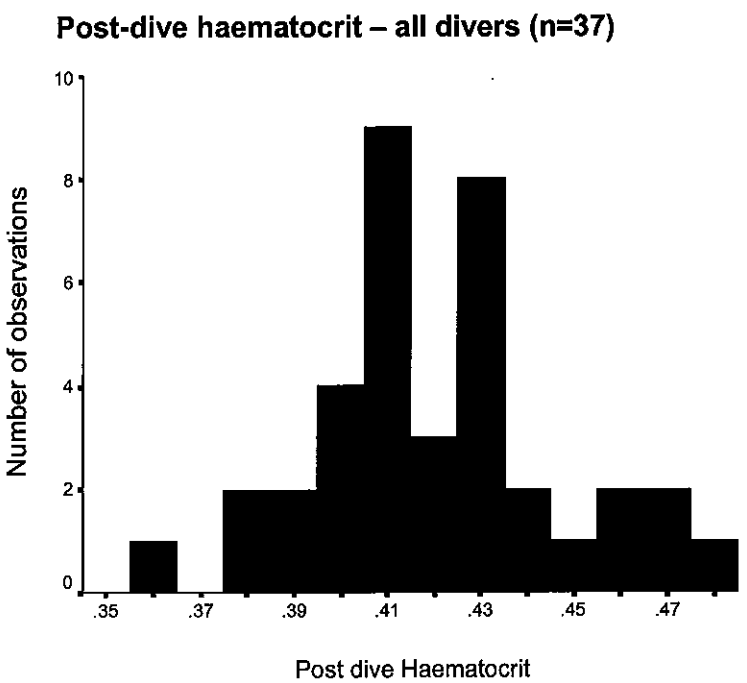
A. Descriptive statistics (n=37)

			Statistic	Std. Error
Pre-dive Haematocrit	Mean		.4132	.00419
	95% Confidence Interval for Mean	Lower Bound	.4047	
		Upper Bound	.4217	
	Std. Deviation		.02550	
	Minimum		.36	
	Maximum		.47	
	Range		.11	
Post dive Haematocrit	Mean		.4205	.00437
	95% Confidence Interval for Mean	Lower Bound	.4117	
		Upper Bound	.4294	
	Std. Deviation		.02656	
	Minimum		.36	
	Maximum		.48	
	Range		.12	

B. Normality tests

This may be shown graphically :





Normality of distribution may be confirmed by the Shapiro-Wilk test :

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Pre-dive Haematocrit	.120	37	.194	.971	37	**.426**
Post dive Haematocrit	.145	37	.049	.966	37	**.317**

Shapiro-Wilk test is not significant and normality of distribution may be assumed for these samples.

C. Paired t-test for pre and post haematocrit - all divers (n=37)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pre-dive Haematocrit - Post dive Haematocrit	-.0073	.00932	.00153	-.0104	-.0042	-4.761	36	**.000**

P < 0.001, indicating overwhelming evidence for a significant change in haematocrit during a dive.

As inclusion levels was evenly balanced between the sexes (n=19 for females and n=18 for males) the sample pairs were analysed by sex in order to ascertain whether haematocrit change was similarly significant between the sexes.

Comparison of pre and post dive haematocrit by sex

A. Descriptive statistics

	Sex			Statistic	Std. Error
Pre-dive Haematocrit	Female (n=19)	Mean		.3984	.00448
		95% Confidence Interval for Mean	Lower Bound	.3890	
			Upper Bound	.4078	
		Std. Deviation		.01951	
		Minimum		.36	
		Maximum		.43	
		Range		.07	
	Male (n=18)	Mean		.4289	.00511
		95% Confidence Interval for Mean	Lower Bound	.4181	
			Upper Bound	.4397	
		Std. Deviation		.02166	
		Minimum		.40	
		Maximum		.47	

		Range		.07	
Post dive Haematocrit	Female (n=19)	Mean		.4042	.00441
		95% Confidence Interval for Mean	Lower Bound	.3949	
			Upper Bound	.4135	
		Std. Deviation		.01924	
		Minimum		.36	
		Maximum		.44	
		Range		.08	
	Male (n=18)	Mean		.4378	.00521
		95% Confidence Interval for Mean	Lower Bound	.4268	
			Upper Bound	.4488	
		Std. Deviation		.02211	
		Minimum		.41	
		Maximum		.48	
		Range		.07	

B. Normality tests

		Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Sex	Statistic	df	Sig.	Statistic	df	Sig.
Pre-dive Haematocrit	female	.141	19	.200(*)	.949	19	**.375**
	male	.202	18	.051	.927	18	**.174**
Post dive Haematocrit	female	.171	19	.145	.960	19	**.578**
	male	.249	18	.004	.905	18	**.069**

Normal distribution of pre and post dive haematocrit samples is indicated for females. In the case of males Kolmogorov-Smirnov is significant in the case of post-dive haematocrit and very close to significance in the case of the pre-dive samples. The small number of samples involved, however, allows acceptance of Shapiro-Wilk as indicative of an acceptable normal distribution.

C. i. Paired t-test for pre and post dive haematocrit : females (n=19)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pre-dive Haematocrit - Post dive Haematocrit	-.0058	.00902	.00207	-.0101	-.0014	-2.799	18	**.012**

P = 0.012, indicating strong evidence of a difference in haematocrit between the pre and post-dive samples for females.

ii. Paired t-test for pre and post dive haematocrit : males (n =18)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pre-dive Haematocrit - Post dive Haematocrit	-.0089	.00963	.00227	-.0137	-.0041	-3.915	17	**.001**

P = 0.001, indicating very strong evidence of a difference between male pre and post dive samples.

To address concerns that the male post dive samples may not have been normally distributed the analysis for males is repeated using Wilcoxon's non-parametric test :

	Post dive Haematocrit - Pre-dive Haematocrit
Z	-3.066(a)
Asymp. Sig. (2-tailed)	**.002**

The test for males remains highly significant, p=0.002

Comparison of degree of change in haematocrit by depth of dive.

As mentioned in the protocol above the lack of prior knowledge of the dive operation made it difficult to plan this section of the study in advance. Nevertheless it was initially hoped that the depth of 10m could be used as a convenient point of division around which to create two groups of 'deep' and 'shallow' divers. Examination of the depths achieved on the dives, though, revealed that there were 3 divers who went to maximum depths within a metre of 10m, and that 2 of these recorded depths of exactly 10m - meaning they could just as easily belong to either deep or shallow group. The figure of 12m therefore suggested itself as a better cut-off depth, with only 1 diver achieving a maximum depth within a metre of this division.

Taking this depth as the cut-off, 23 divers exceeded 12m while 14 stayed at shallower depths. Of the divers who exceeded 12m, 13/23 (57%) showed haemoconcentration, compared to 5/14 (36%) who stayed shallow. Of the divers who showed haemoconcentration, 13/18 (72%) had ventured below 12m and 5/18 (28%) had stayed above this depth.

This simple analysis hints that depth could be associated with an increased tendency to haemoconcentration. But are these results statistically significant ? In the performance of the statistical analysis the use of a paired t-test is now no longer valid. Because each individual diver contributed a pair of blood samples relating to one dive only, a comparison of haematocrit change in deep and shallow divers now involves comparison of two discrete diver groups. In making this analysis, therefore, an Analysis of Variance is employed, with haematocrit and dive depth as two, possibly interacting, factors.

Descriptive statistics (n=37)

	Dive depth	Mean	Std. Deviation	N
Pre-dive Haematocrit	Dive <12m	.4221	.02607	14
	Dive >12m	.4078	.02411	23
	Overall	.4132	.02550	37
Post dive Haematocrit	Dive <12m	.4279	.02887	14
	Dive >12m	.4161	.02463	23
	Overall	.4205	.02656	37

Levene's test for equality of variances

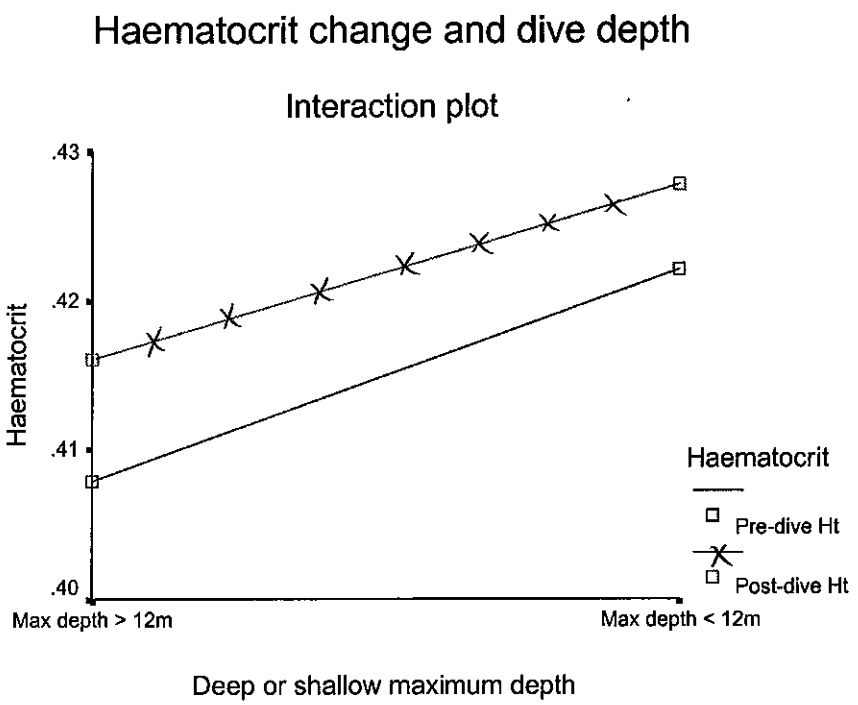
	F	df1	df2	Sig.
Pre-dive Haematocrit	.150	1	35	**.701**
Post dive Haematocrit	.324	1	35	**.573**

Levene's test is not significant, allowing an assumption of normal variance in respect of the pre-dive and post-dive haematocrits for both the deep and shallow groups.

Analysis of variance

		Type III Sum of Squares	df	Mean Square	F	Sig.
Haematocrit change	Sphericity Assumed	.001	1	.001	19.357	**.000**
	Greenhouse-Geisser	.001	1.000	.001	19.357	**.000**
	Huynh-Feldt	.001	1.000	.001	19.357	**.000**
	Lower-bound	.001	1.000	.001	19.357	**.000**
Haematocrit change / Max depth > or < 12m	Sphericity Assumed	2.822E-05	1	2.822E-05	.643	**.428**
	Greenhouse-Geisser	2.822E-05	1.000	2.822E-05	.643	**.428**
	Huynh-Feldt	2.822E-05	1.000	2.822E-05	.643	**.428**
	Lower-bound	2.822E-05	1.000	2.822E-05	.643	**.428**
Error(BLOOD)	Sphericity Assumed	.002	35	4.390E-05		
	Greenhouse-Geisser	.002	35.000	4.390E-05		
	Huynh-Feldt	.002	35.000	4.390E-05		
	Lower-bound	.002	35.000	4.390E-05		

The ANOVA finds overwhelming evidence for a significant difference in the pre-dive and post-dive haematocrits, ($p < 0.001$) as we have already seen above. But there is no evidence of a significant interaction between haematocrit change and depth ($p=0.428$). This may be illustrated graphically :



The plot lines being largely parallel we conclude once again that there is no evidence of a significant interaction. In other words there is no evidence in this study to suggest that divers who dived below 12m had a significantly different degree of haematocrit change compared to those who remained above this depth.

The plot also illustrates the finding that samples from divers who recorded maximum depths less than 12m returned consistently higher haematocrits from both the pre-dive and post-dive samples. Potential reasons for this are discussed below.

Comparison of degree of change in haematocrit by dive duration.

Although this analysis was envisaged in the protocol it turned out that, for operational reasons, divers on the site were issued with strict dive profiles by Coral Cay and tended to make dives of similar durations. Indeed, as we have seen, the standard deviation for the dives studied was only 4.5 minutes. As it was therefore difficult to separate the dives into two meaningful groups representing "long" and "short" dives this analysis was not attempted.

PART FIVE : DISCUSSION

The question of haematocrit change with greater depth of dive deserves further comment. The analysis presented above is based only on maximum depth attained during the dive. Clearly this represents an imperfect tool since, physiologically, one would expect haemo-concentration to depend not just on overall depth reached but also on time spent at that depth. In order to combine these data a dive computer capable of recording a continuous depth-time profile would be needed for each diver and haematocrit change assessed in terms of a single variable reflecting both depth and time exposure. Monitoring of this complexity was beyond the scope of this study.

It is also the case that the maximum depths reached by divers in the >12m group are probably a poor reflection of the depths at which most of the dive was performed. The dive site for this study featured a reef which gave way entirely to sand beyond a depth of about 10m and divers who went deeper than this were generally morning divers who were gaining some depth in order to avoid the possibility of a reverse profile exposure on their second dive later in the day. Having satisfied this requirement they generally would have ascended to continue their diving on the reef itself. It is thus probable that the maximum depths recorded suggest much more severe dive exposures than were actually incurred. It was recognised in the protocol that the study was underpowered for the purpose of testing haematocrit change by depth of dive and, unless the associated haematocrit change was large, would be unlikely to yield a significant result in this respect. A significant result becomes less likely if we also believe this suggestion that the dive profiles experienced by divers in the deep group were in fact much closer in severity to those of their colleagues in the shallow group than is suggested by the bald figures for maximum depth. Even so, and despite these reservations, the finding that amongst the divers who showed haemo-concentration 78% came from the deep group suggest that further investigation with a larger sample size should be performed in this area.

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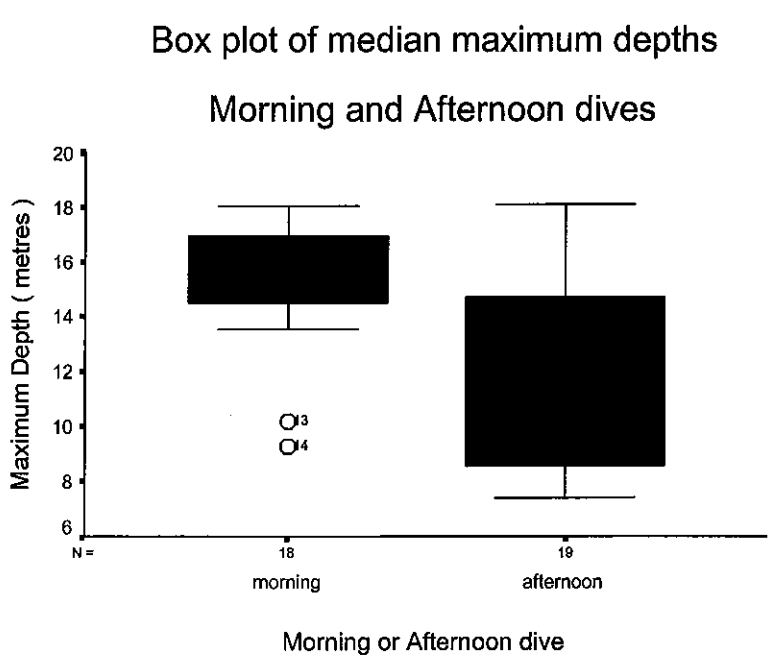
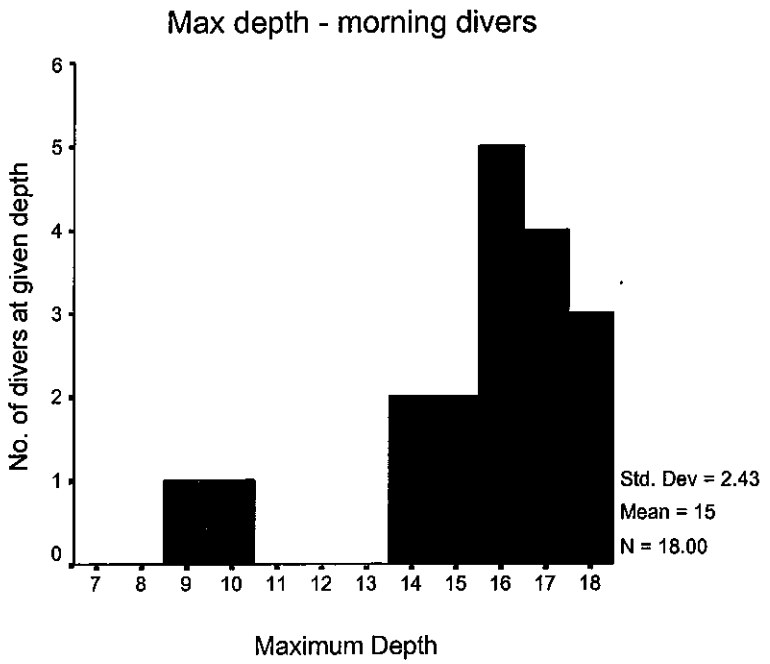
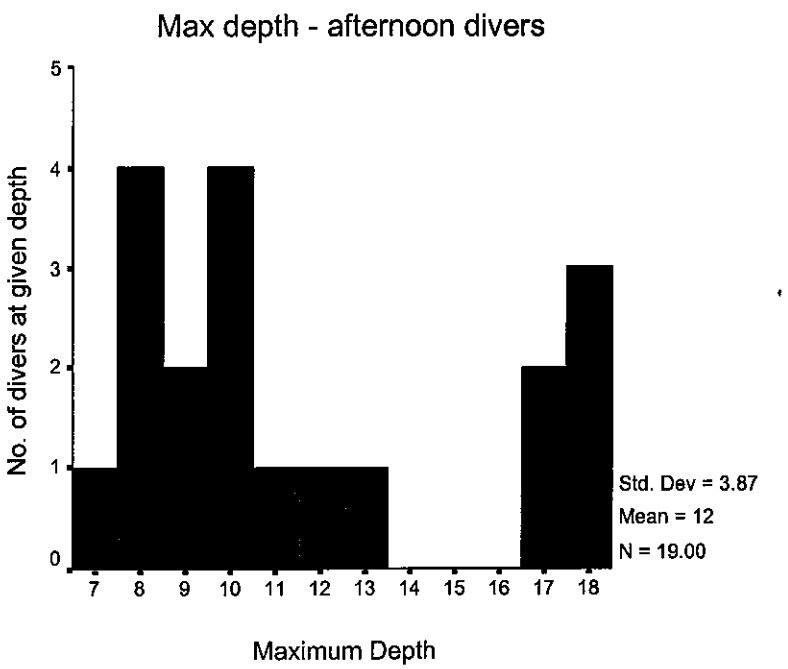
The interaction plot on page 16 illustrated that although the degree of change of haematocrit is similar for both shallow and deep divers the former returned consistently higher haematocrits than the latter, from both the pre-dive and post-dive samples. The explanation for this may well lie with the operational requirement noted above for the deep dives to occur in the mornings whilst afternoon dives – unless the diver were making his first and only dive of the day – were generally shallow.

The extent to which this occurred is illustrated by the following descriptive statistics and plots for maximum depths in the mornings and afternoons:

	Morning/afternoon dive			Statistic	Std. Error
Maximum Depth	morning	Mean		**15.444**	.5718
		95% Confidence Interval for Mean	Lower Bound	14.238	
			Upper Bound	16.651	
		Median		16.200	
		Std. Deviation		2.4259	
		Minimum		9.3	
		Maximum		18.0	
		Range		8.7	
	afternoon	Mean		**11.589**	.8888
		95% Confidence Interval for Mean	Lower Bound	9.722	
			Upper Bound	13.457	
		Median		10.000	
		Std. Deviation		3.8741	
		Minimum		7.4	
		Maximum		18.1	
		Range		10.7	





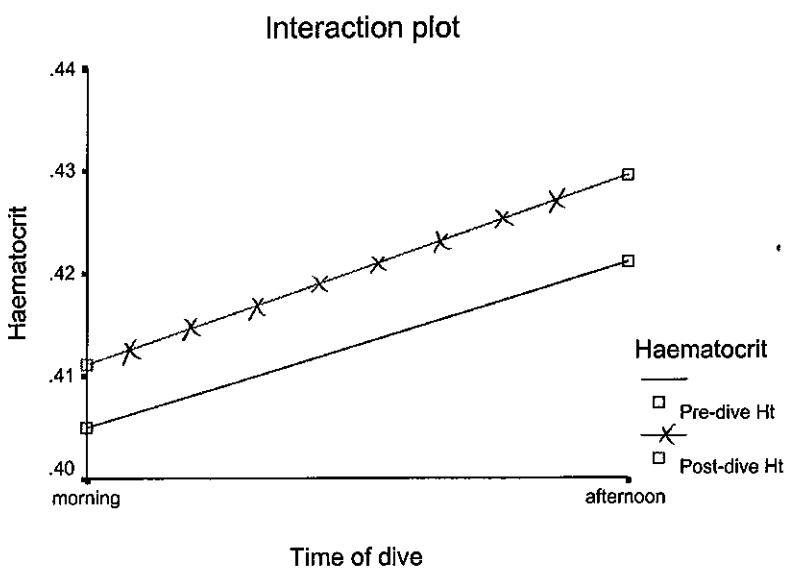


It is likely that the consistently higher haematocrits returned by the shallow divers simply reflects the fact that these divers were operating predominantly in the afternoons and that a morning's activity in the tropical heat, coupled with the dehydrating tendency of a morning dive, more than offset their best efforts at rehydration. The study did not set out specifically to investigate this area, which once again does not allow paired t-test analysis. Given the constraints on time and recruitment already noted it is not surprising that an ANOVA, with haematocrit and time of day as two potentially interacting variables, does not reach significance :

		Type III Sum of Squares	df	Mean Square	F	Sig.
Haematocrit change	Sphericity Assumed	.001	1	.001	22.179	.000
	Greenhouse-Geisser	.001	1.000	.001	22.179	.000
	Huynh-Feldt	.001	1.000	.001	22.179	.000
	Lower-bound	.001	1.000	.001	22.179	.000
Haematocrit / am-pm	Sphericity Assumed	2.466E-05	1	2.466E-05	.560	**.459**
	Greenhouse-Geisser	2.466E-05	1.000	2.466E-05	.560	**.459**
	Huynh-Feldt	2.466E-05	1.000	2.466E-05	.560	**.459**
	Lower-bound	2.466E-05	1.000	2.466E-05	.560	**.459**
Error(BLOODHT)	Sphericity Assumed	.002	35	4.401E-05		
	Greenhouse-Geisser	.002	35.000	4.401E-05		
	Huynh-Feldt	.002	35.000	4.401E-05		
	Lower-bound	.002	35.000	4.401E-05		

No evidence is found of a significant interaction between haematocrit change and time of dive (p = 0.459). The interaction plot has the following appearance :

Haematocrit change and time of dive



The plot is similar to that seen earlier for haematocrit change and depth, which is to be expected as the population of divers is very largely the same. Samples show greater haemo-concentration in the afternoon divers at both the pre-dive and post-dive stages. It could thus be that the blood of divers entering the water for their afternoon dive is already in a more advanced state of haemo-concentration than that of the morning divers as they left the water. Given that repetitive diving is a common setting for a great number of recreational diving accidents, with dehydration implicated as a contributing factor, further work with an adequate sample size could usefully be conducted in an attempt to verify this suggestion.

Finally, we note with regret that the restricted time available for this study prevented the introduction into the study of a shore-based control group. This would have been most useful as a means of quantifying the haematocrit change which could be directly attributed to diving over and above that incurred on land during the same time period. Constraints on the duration of the study, however, made it impossible to generate acceptable statistical power given the number of volunteers available.

PART SIX : SUMMARY

1. The study finds overwhelming evidence for a significant change in haematocrit over the course of a warm water recreational dive (p = <0.001)

mean pre-dive Ht 0.4132 (95% CI 0.4047 - 0.4217)
mean post dive Ht 0.4205 (95% CI 0.4117 - 0.4294)

There is thus no evidence to justify a change of current practice regarding routine rehydration of the recreational diving casualty.

2. Over the course of a recreational dive there is strong evidence of a significant change in haematocrit for females (p = 0.012) and very strong evidence of a change for males (p = 0.001).

3. The study fails to find significant evidence that the degree of haematocrit change is influenced by diving to maximum depths greater or less than 12m, or by diving at different times of the day. The study, however, was underpowered in respect of these analyses and further investigation with an adequate sample size is suggested.

Appendix 1

Consent Form

Haematocrit change in recreational scuba divers following single dive exposure

Patient Number for this trial :

Name of Researcher: **Simon Williams**

Please initial box

1. I confirm that I have read and understand the information sheet dated....., version....., for the above study ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected ☐
3. I agree to take part in the above study. ☐

Name of patient	Date	Signature
Researcher	Date	Signature

Appendix 2

Taking Part In Medical Research
General Information for Volunteers

You are being invited to take part in a research project. Here is some general information to help you decide whether or not to take part. Please take time to read the following information carefully. Ask us if there is anything you do not understand or if you would like more information and please take time to decide whether or not you wish to take part. Thank you.

You will not receive any direct benefit from taking part in the study. However, information obtained during the course of the study may help us to understand better the physiological changes which affect recreational scuba divers during a dive. It may also help us in selecting treatment for future divers who develop medical problems as a result of diving .

It is up to you to decide whether to take part or not. If you do decide to take part you will be given an information sheet and consent form. Even if you do decide to take part, you are free to withdraw at any time and without giving a reason. This will not affect the standard of medical care you will receive. Your doctor will not be upset if you decide not to take part.

All the information collected about you during the course of the research will be kept strictly confidential. Any published report of the research will not identify you.

Your GP will not be informed that you are taking part. If this is problem for you, you should discuss it with your study researcher.

Taking part in the study will not affect your diving insurance, nor your ability to obtain insurance in the future.

Consumers for Ethics in Research (CERES) publish a leaflet entitled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, London N16 0BW.

Appendix 3

Taking Part In Medical Research
Project information

Dear Volunteer,

You are invited to take part in a medical research project. Here is some information on the project to help you decide whether to take part. Please take the time to read it and ask if there is anything you do not understand or would like clarified. Your participation is entirely voluntary.

Haematocrit Change in Recreational Scuba Divers following Single Dive Exposure.

Purpose of this study : To gain an understanding of the extent to which recreational scuba divers become dehydrated over the course of a dive.

It is known that a diver's body undergoes a series of automatic adjustments in response to the underwater environment of a dive. One such adjustment concerns the redistribution of fluids within the body during a dive. This may be a cause of dehydration in the diver, though the extent to which this occurs, if at all, is not known at present. This study aims to gain an understanding of this process by measuring haematocrit. This is the ratio of cells to plasma in the circulating blood and is a good indicator of hydration.

Recruitment of volunteers : This study is being carried out on the Coral Cay Conservation site in Fiji. All volunteer divers attending the site will be invited to take part. The researchers are funding the project themselves and are not in receipt of funds from any outside agency. The aim is to recruit at least 30 participants to the study. No remuneration or other incentive will be offered to any volunteer taking part.

Who is organising the study ? : The study is being performed under the auspices of the Diving Diseases Research Centre in Plymouth, U.K., which is also the medical advisory unit for Coral Cay Conservation. Recruitment and testing of volunteers will be carried out over three months between February and May 2004, with data analysis being performed over the following summer.

What will happen to me if I take part ? : If you decide to participate your involvement will be to give a blood test immediately before and after one dive. This will be collected by the standard method from a needle puncture to a vein in the arm. You will be asked to provide samples relating to one dive only; there will be no repeat testing of volunteers. Your samples will then be analysed for haematocrit measurement. Your blood will be tested for this parameter only - no other testing will take place and your blood sample will be disposed of after the test.

Are there any risks in taking part ? : After the samples are taken you may experience some local bruising of the arm around the needle puncture site - as is normal whenever blood samples are taken. Other than this there are no side effects envisaged as a result of participation in the study.

Is the doctor being paid for including me in the study ? : Neither your General Practitioner nor the doctor on site, nor any other person or organisation will receive any payment as the result of your participation in this study.

Are there any restrictions on what I may eat or do ? : Blood samples will be taken as soon as practically possible before and after the dive. We ask volunteers not to drink between the tests. Other than this your daily activities are not affected in any way.

Confidentiality - who will know that I am taking part in the study ? : No access to your medical records will be made and no information will be transmitted to your General Practitioner as a result of your involvement in this study. The information which is collected about you during the course of this research will be kept strictly confidential and the results of your blood test will be rendered anonymous.

GP notification : No communication will be made with your General Practitioner.

Ethics Approval : Ethical approval to pursue this research has been granted by the Torbay Local Research Ethics Committee, South Devon Healthcare Trust, Hengrave House, Torbay Hospital, Lawes Bridge, Torquay TQ2 7AA. United Kingdom

What will happen to the results of the study ? : The aim of the researchers is to publish their findings in a medical journal over the coming year.

Further information : Any further enquiries may be addressed to Dr. Simon Williams, on-site Medical Officer for Coral Cay, or to Dr. Phil Bryson, Diving Diseases Research Centre, Tamar Science Park, Research Way, Plymouth PL6 8BU

Appendix 4 :
Raw Data

Date	Volunteer	Age / Sex	Sample 1 time	Descent Time	Delay (mins)	Surface Time	Sample 2 time	Delay (mins)	Maximum Depth (m)	Duration of dive (mins)	Pre-dive Ht	Post-dive Ht	Change
02.03.04	1	F 22	1532	1547	15	1631	1641	10	12.6	44	0.41	0.41	0
04.03.04	2	M 27	1547	1601	14	1645	1658	13	10	44	0.4	0.41	0.01
	3	M 18	1549	1602	13	1646	1655	9	9.8	44	0.43	0.43	0
	4	M 26	1546	1601	15	1645	1700	15	10	44	0.45	0.48	0.03
	5	F 19	1550	1602	12	1646	1647	11	8.9	44	0.43	0.43	0
06.03.04	6	M 19	1502	1523	21	1606	1614	8	16.4	43	0.37	0.4	0.03
	7	M 18	1506	1523	17	1558	1612	14	18	35	0.42	0.43	0.01
	8	M 19	1504	1523	19	1606	1618	12	16.8	43	0.45	0.46	0.01
	9	M 19	1508	1523	15	1558	1611	13	18.1	35	0.4	0.43	0.03
08.03.04	10	M 24	1400	1411	11	1448	1456	8	16.7	37	0.43	0.44	0.01
	11	M 18	1407	1423	15	1503	1512	9	7.4	40	0.43	0.43	0
	12	M 18	1415	1427	12	1510	1522	12	7.5	43	0.41	0.42	0.01
	13	F 23	1430	1447	17	1526	1543	17	17.7	39	0.39	0.41	0.02
09.03.04	14	M 18	924	943	19	1023	1034	11	10.2	40	0.4	0.41	0.01
	15	M 18	926	943	17	1023	1032	9	9.3	40	0.47	0.47	0
10.03.04	16	F 18	914	930	16	1005	1015	10	16.5	35	0.38	0.41	0.03
	17	F 19	917	932	15	1015	1031	16	16.4	43	0.4	0.4	0
	18	F 19	1020	1037	17	1111	1120	9	17.2	33	0.43	0.44	0.01
	19	M 18	1106	1117	11	1200	1209	9	16.7	43	0.46	0.42	-0.04
	20	F 21	1025	1036	11	1120	1130	10	15.4	44	0.4	0.4	0
	21	F 19	1108	1117	9	1200	1211	11	16.4	43	0.41	0.41	0
15.03.04	22	F 19	1100	1120	20	1201	1215	14	17.9	41	0.36	0.36	0
	23	F 22	1056	1120	24	1201	1220	19	17	41	0.41	*Haem*	*Haem*
18.03.04	24	F 24	934	948	14	1028	1038	10	18	40	0.39	0.4	0.01
	25	F 27	1034	1051	17	1133	1147	14	16	42	0.41	0.41	0
	26	F 18	1455	1510	15	1545	1601	16	8.2	35	0.39	0.39	0
24.03.04	27	M 21	1014	1029	25	1015-	1034	19	15.7	36	0.43	0.43	0
	28	F 19	1018	1039	21	1017	1037	20	15.7	38	0.4	0.42	0.02
	29	M 19	1355	1410	15	1446	1458	12	12.3	36	0.46	0.47	0.01
05.04.04	30	M 26	1550	1610	20	1640	1654	14	11.2	30	0.42	0.42	0
	31	M 18	1553	1610	17	1652	1700	8	8.4	42	0.46	0.46	0
	32	M 19	1556	1615	19	1702	1712	10	8.3	47	0.43	0.45	0.02
06.04.04	33	F 21	1031	1049	18	1119	1133	14	14	30	0.43	0.43	0
	34	F 25	1108	1130	22	1210	1225	15	17.8	40	0.41	0.41	0
	35	F 19	1112	1130	18	1210	1220	10	16.9	40	0.39	0.4	0.01
	36	F 19	1600	1613	13	1649	1700	11	9.5	36	0.38	0.38	0
	37	M 33	1630	1641	11	1709	1720	11	8.8	28	0.41	0.41	0
	38	F 19	1102	1116	14	1156	1216	20	16.6	40	0.37	0.38	0.01
	39	M 18	1105	1121	16	1203	1220	17	14.5	42	0.42	0.43	0.01
	40	F 24	1109	1130	21	1215	1224	9	13.5	45	0.39	0.39	0
	41	F 20	1528	1543	15	1418	1427	9	8.8	35	0.35	0.36	0.01

Acknowledgements

In pursuing this project the researchers would like to acknowledge the support of the very many people without whose help the work could not have been completed. Especial thanks are extended to Dr. Phil Bryson at the Diving Diseases Research Centre, Plymouth, without whose backing the project would not have got off the ground, and to the laboratory staff at Torbay Hospital, whose tolerant instruction enabled me to master some basic, practical haematology. The biggest thanks, though, are reserved for the staff of Coral Cay Conservation, in particular James Sawyer at head office, who obtained permission for the research to go ahead at the Ravinake site, the local staff under Pete Corson and Henry Milner who made it possible to fit the project into the daily diving round, and to the divers themselves, virtually all of whom came forward with unanticipated eagerness to participate in the trial. I hope the results justify the support that everyone has shown.